subjecting the removed sensor domain to gene reshuffling, ligating the mutated sensor domain into the DNA encoding the regulatory protein, and testing the bacteria for enhanced response to said phenols and substituted phenols over the response thereto for wild-type bacteria without altering other domains.

a2 cont.

REMARKS

Claims 1-7 are pending in the application. Claims 2 - 7 have been canceled, claim 1 has been amended and Claim 8 has been added. In the Office Action dated June 30, 2001, the Examiner stated that the Declaration is defective because: corrections made to the post office addresses of C. Kuske and T. Terwilliger have not been initialed as required. Applicants agree to submit a corrected Declaration as soon as allowable subject matter is identified.

The Examiner continued by stating that the drawings submitted with this application have not been reviewed by a draftsperson at this time and that when formal drawings are submitted, the draftperson will perform a review. Applicants further agree to submit formal drawings as soon as allowable subject matter is identified.

The Examiner then stated that the present application contains sequence disclosures that are encompassed by the definitions for nucleotide and/or amino acid sequences set forth in 37 CFR 1.821(a)(l) and (a)(2); however, the application fails to comply with the requirements of 37 CFR 1.821 through 1.825; applicants' attention is directed to the final rulemaking notice published at 55 FR 18230 (May 1, 1990), and 1114 OG 29 (May 15, 1990). Applicants agree to provide an initial computer readable form (CRF) copy of the "Sequence Listing", an initial paper copy of the "Sequence Listing", as well as an amendment directing its entry into the specification, and a statement that the content of the paper and CRF copies are the same and, where applicable, include no new matter, as well as to provide return a copy of the attached Notice to Comply with the response, as soon as allowable subject matter is identified.

The Examiner next objected to the drawings because of the following informalities: the y-axes of Figures 2-7 are not labeled. For all graphs, units are "β-galactosidase activity normalized for time length of assay and number of cells in the assay" as stated in lines 8-9 on page 11 of the subject Specification, as originally filed. The addition of the ordinates for all of the objected to Figures will be added when formal drawings are submitted.

Claim 3 was objected to because of the recitation of "DmpR, MopR, PhhR, PhlR, XylR, and TbuT", since abbreviations, unless otherwise obvious should not be recited in the claims without at least once reciting the entire phrases corresponding to the abbreviations. Applicants respectfully disagree with the Examiner on this ground of objection, since the abbreviations objected to by the Examiner are recited in all of the references cited by the Examiner as members of the NtrC family of regulatory proteins and, in fact, are commonly used throughout the literature.

Claims 1, 5 and 6 were rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicants regard as the invention, since claim 1 is confusing in that it is unclear as to what activities are in the "sensor domain" and which are in the "other domains"; there must be at least the following "independent activities": an organic molecule binding domain, a DNA binding domain, and a transcriptional activation domain. It was suggested that applicants clearly identify the "independent activities" of the "sensor domain" and the "other domains". Additionally, claim 1 recites the limitation "A method for enhancing the response" (emphasis added), and the Examiner stated that there is insufficient antecedent basis for this limitation in the claim. The Examiner suggested that the term be replaced with, for example, " A method for enhancing a response". The Examiner continued by stating that the terms "enhanced" and "enhanced response" in claims 1, 5, and 6 are unclear absent a statement defining to what the response to the organic molecule is being compared. Applicants wish to thank the Examiner for having identified this difficulty and for suggesting alternative language for claims 1, 5, and 6 in order that applicants more particularly point out and distinctly claim their invention, and have

modified claim 1 in order to reflect these suggestions. No new matter has been added by these changes.

Claims 1- 7 were then rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors, at the time the application was filed, had possession of the claimed invention, since claims 1 (claims 2, 3, and 7 dependent thereon) and 4 (claims 5 and 6 dependent thereon) are directed to a method of enhancing the response of bacteria to organic molecules, said method utilizing a genus of: organic molecules, regulatory proteins, and genes encoding metabolic enzymes. The specification teaches only a single representative species of such organic molecules, i.e., phenols and six representative species of regulatory proteins, i.e., DmpR, MopR, PhhR, PhlR, XylR, and TbuT. Moreover, the specification fails to describe any other representative species by any identifying characteristics or properties other than the "functionality" of being organic molecules or regulatory proteins.

The Examiner states further that the present Specification is enabling for a method of enhancing the expression of a reporter gene under the control of a P_o promoter in *Pseudomonas* and *Acinetobacter* bacteria in response to the presence of phenols, said bacteria expressing a regulatory protein from the group consisting of DmpR, MopR, PhhR, PhlR, XylR, and TbuT with a sensor domain that binds phenols resulting in binding of the regulatory protein to a P_o promoter and activation of said reporter gene, said method comprising mutating the sensor domain of the regulatory protein by mutagenic PCR or gene shuffling, but does not provide enablement for a method of enhancing any response of any bacteria to any organic molecules, said bacteria having any regulatory protein with any sensor domain that binds to any cognate promoter sequence and activates expression of any genes encoding metabolic enzymes, said method comprising performing any modification to the sensor domain of the regulatory protein such that the response to the organic molecules is enhanced without altering the other domains, and optionally wherein the step of modifying is achieved by any mutation. In response to the Examiner's

arguments, applicants have amended claim 1 to limit the organic molecules to phenols and substituted phenols, to specify the bacteria as *Pseudomonas putida* and *Acinetobacter* bacteria and the regulatory proteins as selected from the group consisting of DmpR, MopR, PhhR, PhlR, XylR, and TbuT. Applicants have also added *Escherichia coli* to claim 1, since the EXAMPLE in the subject Specification, as originally filed, demonstrates that *E. coli* engineered in accordance with the teachings of the present invention gives the expected results. No new matter has been added by these changes since the Examiner has stated that adequate support therefor may be found in the subject Specification, as originally filed, and E. coli is discussed in the EXAMPLE as stated.

Claims 1-4 and 7 were next rejected under 35 U.S.C. 102(b) as being anticipated by Shingler et al. (J. Bacteriol. 176:7550- 7557), since the Examiner asserts that Shingler et al. teaches that DmpR belongs to the NtrC family of transcriptional activators and shares significant sequence similarity with XylR, a Pseudomonas regulator of toluene and xylene catabolism (p 7550, Introduction, paragraph 1) and that DmpR responds to (methyl)phenols with the magnitude of transcriptional response differing depending on the position of the methyl substituent (p 7550, abstract). Shingler et al. also teaches that DmpR and XylR are regulatory proteins composed of distinct functional domains (p 7550, Introduction) and that the A domains of these proteins bind aromatic compounds resulting in transcriptional activation (p 7556, paragraph 2). Shingler et al. further teaches a method of mutating DmpR by chemical mutagenesis using ethyl methanesulfonate as mutagen (pp 7551-7552 under Construction of P_o Km selection strain and isolation of DmpR specificity mutant) to generate a mutant DmpR that, when expressed in *Pseudomonas putida* with a chromosomally inserted reporter gene (p 7552, under Construction of P_o luxAB reporter strain and luciferase assays), exhibits increased luciferase expression relative to wild-type DmpR in response to 4-methylphenol, 3,4-dimethylphenol, and 4- ethylphenol (p 7554, Fig 3) and that sequencing the gene encoding the DmpR mutant revealed a mutation at codon 135 (p 7554, under Genetic selection of an effector specificity mutant, DmpR-EI35K) of the A domain of

DmpR (amino acids 1-211; p 7556, paragraph 2). Shingler et al. also teaches that a comparison of the responses of the wild-type and mutant DmpR to various phenolic derivatives suggests that, in addition to the increased responses of the mutant to 4-methylphenol, 3,4-dimethylphenol, and 4-ethylphenol, the mutant DmpR mediates responses to phenol, 2-methylphenol, and 3-methylphenol to similar extents as wild-type DmpR (p 7554, under Effector profile comparison of DmpR+ and DmpR-EI35K), suggesting that the mutant DmpR response to 4-methylphenol, 3,4-dimethylphenol, and 4-ethylphenol is enhanced relative to wild-type DmpR without altering the function of the other (DNA binding and transactivation) domains. This anticipates claims 1-4 and 7 as written. Applicants respectfully disagree with the Examiner concerning this ground of rejection.

Applicants have amended claim 1, deleted claims 2-7 and added claim 8 to more clearly distinguish Shingler et al.; that is, the limitations of originally filed claims 5 and 6 have been added to claim 1 and newly added claim 8. No new matter has been added by these changes and additions. In the Abstract (p. 7550) of Shingler et al. it is stated that: "Experiments involving an elevated copy number of the dmp system demonstrate that growth on para-substituted methylphenols is limited by the level of catabolic enzymes. ... The single-point mutation in DmpR-E135K, which results in a Glu-to-Lys change in residue 135 also results in a regulator with enhanced recognition of para-substituted methylphenols. The DmpR-E135K mutation, when introduced into the wild-type strain, confers enhanced utilization of the para-substituted methylphenols." Thus, Shingler et al. teaches only single-point mutation using ethyl methane sulfonate and elevated numbers of the dmp system. Such mutations could also have produced a lower utilization of the methylphenols. In view of newly added claim 8 and amended claim 1, applicants believe that the Shingler et al. reference does not anticipate the present claimed invention; rather, the present claimed invention teaches a systematic procedure for selecting those mutations from a random set of mutations which improve response to phenols and substituted phenols over that for the wild type.

The bacteria and regulator proteins of claims 2 and 3 not addressed in the 35 U.S.C. 102(b) rejection above were next rejected by the Examiner under 35 U.S.C. 103(a) as being unpatentable over Shingler et al. in view of any of Willardson et al. (Appl. Environ. Microbiol. **64**: 1006-1012), Schirmer et al. (J Bacteriol. **179**:1329-1336), Ng et al. (J Bacteriol. **177**:1485-1490), Burchhardt et al. (Mol. Gen. Genet. **254**:539-547), or Byrne et al. (J. Bacteriol. **178**:6327-6337). Applicants have canceled claims 2 and 3 and believe that no further response is required to this ground of rejection.

Claims 5 and 6 were then rejected under 35 U.S.C. 103(a) as being unpatentable over Shingler et al. in view of Willardson et al. and either of Cadwell et al. ("Mutagenic PCR" pp 583-589 in "PCR Primer, A Laboratory Manual", Cold Spring Harbor Laboratory Press, 1995) or Stemmer (Nature 370:389-391), since Shingler et al. discloses the teachings described above; however, Shingler et al. does not teach methods of enhancing bacterial response to organic molecules by mutating the gene encoding DmpR using mutagenic PCR or gene shuftling. The Examiner asserted that Willardson et al. discloses a biosensor using *Escherichia coli* expressing XylR that responds to toluene and derivatives thereof by luminescence proportional to the concentration of toluene or derivatives thereof present in a medium. Willardson et al. further teaches that "the development of this biosensor for toluene and its derivative compounds demonstrates the feasibility of constructing similar biosensors with specificity for other organic contaminants by using their corresponding transcriptional activators" (p 1011, bottom- p 1012, top). Willardson et al. also suggests using other bacterial strains as biosensors (p 1012, top). The Examiner continued by stating that Cadwell et al. teaches a mutagenic PCR method of randomly mutating a nucleic acid in order to generate a library of mutant nucleic acids (p 584 under Protocol). Cadwell et al. further teaches that using these mutants, one can apply a screening method to isolate individual clones that exhibit a particular property. (p 583, Introduction, paragraph 2). Additionally, the Examiner stated that Stemmer et al. teaches a method of *in vitro* homologous recombination of pools of selected mutant genes by random fragmentation and PCR

reassembly; that is, gene shuffling (p 390, Fig 1) and teaches that one would use gene shuffling over mutagenic PCR because mutagenic PCR is not combinatorial and thus, is more limited in the number of possible mutations (p 389, abstract and p 390, right column).

The Examiner concluded that it would have been obvious to one having ordinary skill in the art at the time of the invention to mutate only the sensor domain; that is, the A domain as described by Shingler et al. because one having ordinary skill would have recognized that, in order to broaden the binding specificity of the sensor domain, one could mutate only the protein domain responsible for binding the effector compound. This is, the sensor domain and not the DNA binding or transactivation domains of DmpR and XylR. Therefore, the Examiner concludes, it would have been obvious to one having ordinary skill in the art to combine the teachings of Shingler et al., Willardson et al. and either of Cadwell et al. or Stemmer for a method of mutating the sensor domain of a regulatory protein by mutagenic PCR or gene shuffiing. One would have a reasonable expectation of success for a method of enhancing the response of bacteria to organic molecules by mutating the sensor domain of a regulatory protein by mutagenic PCR or gene shuffling because of the results of Shingler et al. Therefore, claims 5 and 6, drawn to a method of enhancing the response of bacteria to organic molecules by mutating the sensor domain of a regulatory protein by mutagenic PCR or gene shuffling would have been obvious to one having ordinary skill in the art. Applicants respectfully disagree with the Examiner concerning this ground of rejection.

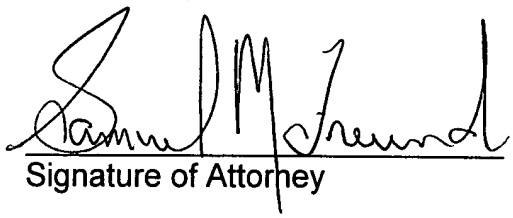
Turning now to the rejection of claims 5 and 6 under 35 U.S.C. 103(a), applicants fail to understand the Examiner's combination of Willardson et al., Cadwell et al. and Shingler et al. in view of the comments made by the Examiner in the subject Office Action. On page 6 of the Office Action, the Examiner stated: "Since the amino acid sequence of a protein determines it structural and functional properties, predictability of which changes can be tolerated in a protein's amino acid sequence and obtain the desired activity requires a knowledge of an guidance with regard to which amino acids in the protein's sequence, if any, are tolerant of

modification and which are conserved, and detailed knowledge of the ways in which the protein's structure relates to its function.". Moreover, on page 7 of the Office Action, the Examiner stated: "While recombinant and mutagenesis techniques are known, it is not routine in the art to screen for multiple substitutions or multiple modifications, as encompassed by the instant claims, and the positions within a protein's sequence where amino acid modifications can be made with a reasonable expectation of success in obtaining the desired activity/utility are limited in any protein and the result of such modifications is unpredictable. In addition, one skilled in the art would expect any tolerance to modification for a given protein to diminish with each further and additional modification, e.g. multiple substitutions.". From these statements, applicants believe that there would be no motivation to combine the references in the manner done by the Examiner, since the possibility of success would be small. Although the possibility of success in mutating the sensor domain might not be large, the present invention teaches a method for rapidly screening the resulting mutations. Therefore, applicants believe that the Examiner has failed to meet the burden of providing a *prima facie* case for obviousness under 35 U.S.C. 103(a).

For the reasons set forth hereinabove, applicants believe that claims 1 and 8 as amended are in condition for allowance, and such action by the Examiner at an

early date is earnestly solicited. Reexamination and reconsideration are respectfully requested.

Respectfully submitted,



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MARKED-UP VERSION OF THE AMENDED CLAIM

1(Amended). A method for enhancing [the] a response of bacteria selected from the group consisting of *Pseudomonas putida*, *Acinetobacter* and *Escherichia coli* to [organic molecules] phenols and substituted phenols over the response exhibited by wild-type bacteria of the same strain, said bacteria having a regulatory protein selected from the group consisting of DmpR, MopR, PhhR, PhlR, XylR, and TbuT with discrete functional domains for independent activities[, one such domain being] including a sensor domain that detects said [organic molecules] phenols and substituted phenols through a direct physical interaction forming a protein-molecule complex which binds to a cognate promoter sequence and activates expression of genes encoding metabolic enzymes, a DNA-binding region, and a transcription activation region, said method comprising [modifying] the steps of removing the sensor domain from the bacterial DNA encoding the regulatory protein, subjecting the removed sensor domain to mutagenic polymerase chain reaction, ligating the mutated sensor domain into the DNA encoding the regulatory protein, and testing the bacteria for enhanced response to said [organic molecules] phenols and substituted phenols over the response thereto for wild-type bacteria [the sensor domain of the regulatory protein such that the response to the organic molecule is enhanced] without altering other domains.